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Title of the invention Therapeutic use

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THERAPEUTIC USE

This invention relates to new uses for polynucleotides and polypeptides encoded by them, to their use in therapy and to agonists, antagonists and/or inhibitors thereof which are useful in therapy.

In particular, the present invention relates to the vanilloid 2 receptor (hereinafter VR2) polypeptide, otherwise known as TRPV2, VRL, VRL-1, Vanilrep2, VRCC, VRRP-1, GRC or SAC2b.

Several patent applications describing cDNA encoding human VR2 have been published: WO 99/37675 (published 29 July 1999), WO 99/37765 (published 29 July 1999), WO 99/46377 (published 16 September 1999), WO 00/22121 (published 20 April 2000), GB-2,346,882 (published 23 August 2000), WO 01/34805 (published 17 May 2001), WO 01/46258 (published 28 June 2001) and EP-1 160 254 (published 12 December 2001).

More particularly, the present invention relates to new uses of the VR2 polypeptide, to new uses for compounds which modulate the activity of a VR2 polypeptide, to new uses of a polynucleotide encoding a VR2 polypeptide, and to new uses of antisense polynucleotides to a polynucleotide encoding a VR2 polypeptide. Such uses include the treatment of disorders associated with the function (or misfunction) of vasopressin and/or oxytocin, including water retention, lactatory activity and modulation of uterine contraction in the female, and erectile function in the male. A further use includes the treatment of schizophrenia. In a further aspect, the invention relates to methods for treating conditions associated with VR2 imbalance or mutation, with the compounds which modulate the activity of a VR2 polypeptide, for example, as agonists, antagonists and/or inhibitors thereof.

Most especially, the present invention relates to the treatment or prevention of pre-term labour, erectile dysfunction and/or hypertension, and associated disorders, as well as schizophrenia. Even more especially,

the present invention relates to the treatment or prevention of pre-term labour, erectile dysfunction and/or hypertension and associated disorders.

The present invention is based on the surprising finding that VR2 is expressed at significantly higher levels in certain regions of the CNS than in a wide range of other regions and tissues tested. In particular, localisation is in the hypothalamus, and predominantly in the paraventricular and supraoptic nuclei.

Figure 1 shows the nucleic acid sequence (coding region of SEQ ID NO: 1) and the predicted amino acid sequence (SEQ ID NO: 2) for VR2.

Figure 2 shows the results of single-label colorimetric immunohistochemistry showing localisation of VR2 immunoreactivity in primate supraoptic nucleus (SON) and paraventricular nucleus of the hypothalamus (PVN).

Figure 3 shows the results of single-label colorimetric immunohistochemistry showing localisation of VR2 immunoreactivity in primate pituitary and suprachiasmatic nucleus.

Figure 4 shows the results of immunofluorescence microscopy showing regional co-expression of VR2, oxytocin and vasopressin in primate hypothalamic paraventricular nucleus.

Figure 5 shows the results of immunofluorescence microscopy showing regional co-expression of VR2, oxytocin and vasopressin in primate supraoptic nucleus (SON).

Thus in a first aspect, the present invention relates to the use of a compound selected from:

(a) a VR2 polypeptide;

(b) a compound which modulates the activity of a VR2 polypeptide;

(c) a polynucleotide encoding a VR2 polypeptide; or

(d) an antisense polynucleotide to a polynucleotide encoding a VR2 polypeptide,

for the manufacture of a medicament for treating pre-term labour, erectile dysfunction and/or hypertension, and associated disorders, or schizophrenia. Particularly the use is for treating pre-term labour, erectile dysfunction and/or hypertension, and associated disorders.

In an alternative aspect of the present invention, there is provided a method for the treatment of pre-term labour, erectile dysfunction and/or hypertension, and associated disorders or schizophrenia which comprises administration of an effective amount of a compound selected from:

(a) a VR2 polypeptide;

(b) a compound which modulates the activity of a VR2 polypeptide;

(c) a polynucleotide encoding a VR2 polypeptide; or

(d) an antisense polynucleotide to a polynucleotide encoding a VR2 polypeptide,

to a patient in need of such treatment. In particular the method is for the treatment of pre-term labour, erectile dysfunction and/or hypertension, and associated disorders.

Compounds which modulate the activity of a VR2 polypeptide include compounds that activate the VR2 polypeptide and also compounds which inhibit the activity of a VR2 polypeptide. Compounds which inhibit the activity of a VR2 polypeptide are particularly preferred.

VR2 polypeptides for use in the invention include isolated polypeptides comprising an amino acid sequence which has at least 95% identity, preferably at least 97 to 99% identity, to that of SEQ ID NO: 2. Such polypeptides include those comprising the amino acid of SEQ ID NO: 2.

Further, VR2 polypeptides include isolated polypeptides in which the amino acid sequence has at least 95% identity, preferably at least 97 to 99% identity, to the amino acid sequence of SEQ ID NO: 2. Such polypeptides include the polypeptides of SEQ ID NO: 2.

Still further, VR2 polypeptides include isolated polypeptides encoded by a polynucleotide comprising the sequence contained in SEQ ID NO: 1.

The VR2 polypeptides may be in the form of the "mature" protein or may be a part of a larger protein such as a precursor or a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The VR2 polypeptides can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

For preparing VR2 polypeptides by recombinant means, a polynucleotide encoding a VR2 polypeptide can be used (hereinafter a "VR2 polynucleotide").

VR2 polynucleotides may be obtained, using standard cloning and screening techniques (Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y.(1989) and United Kingdom patent publication No. 2,346,882 in the name of Merck Sharp & Dohme Limited) from a cDNA library derived from mRNA in cells of human brain. VR2 polynucleotides can also be obtained from natural sources such as genomic DNA libraries or can be synthesised using well-known and commercially available techniques.

GB-A-2,346,882 further discloses methods for the recombinant production of VR2 polypeptides, including expression vectors and hosts and details of purification methods.

VR2 polypeptides or their fragments or analogs thereof, or cells expressing them, can also be used as immunogens to produce antibodies immunospecific for polypeptides of the present invention. The term

"immunospecific" means that the antibodies have substantially greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against VR2 polypeptides may be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a non-human animal, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used.

Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) **256**:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, *Immunology Today* (1983) 4:72) and the EBV-hybridoma technique (Cole *et al.*, Monoclonal Antibodies and Cancer Therapy, 77-96, Alan R. Liss. Inc., 1985).

Techniques for the production of single chain antibodies, such as those described in US Patent No. 4,946,778, can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms, including other mammals, may be used to express humanised antibodies.

Antibodies against polypeptides of the present invention may be employed to treat pre-term labour, erectile dysfunction and/or hypertension, and associated disorders or schizophrenia in accordance with the present invention. Antibodies may particularly be employed to treat pre-term labour, erectile dysfunction and/or hypertension, and associated disorders.

VR2 polypeptides can be used to devise screening methods to identify compounds which modulate the activity of said VR2 polypeptides. Such modulators include compounds which stimulate (agonists) or inhibit (antagonists) the function of the VR2 polypeptides. In general, modulators of VR2, such as agonists or antagonists, may be employed for therapeutic and prophylactic purposes for pre-term labour, erectile dysfunction and/or hypertension, and associated disorders or schizophrenia, and particularly

for pre-term labour, erectile dysfunction and/or hypertension, and associated disorders. Antagonists (or inhibitors) of VR2 are particularly preferred. Compounds may be identified from a variety of sources, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. Such modulators so-identified may be natural or modified substrates, ligands or receptors of the VR2 polypeptides; or may be structural or functional mimetics thereof (see Coligan *et al.*, Current Protocols in Immunology 1(2): Chapter 5 (1991)).

The screening method may simply measure the binding of a candidate compound to the VR2 polypeptides, or to cells or membranes bearing the VR2 polypeptide, or a fusion protein thereof by means of a label directly or indirectly associated with the candidate compound. Alternatively, the screening method may involve competition with a labelled competitor. Further, these screening methods may test whether the candidate compound results in a signal generated by activation or inhibition of the VR2 polypeptides, using detection systems appropriate to the cells bearing the VR2 polypeptide. Inhibitors of activation are generally assayed in the presence of a VR2 agonist, and the effect on activation by the agonist by the presence of the candidate compound is observed. Constitutively active polypeptides may be employed in screening methods for inverse agonists or inhibitors, in the absence of an agonist or inhibitor, by testing whether the candidate compound results in inhibition of activation of the VR2 polypeptide. Further, the screening methods may simply comprise the steps of mixing a candidate compound with a solution containing a VR2 polypeptide to form a mixture, measuring VR2 activity in the mixture, and comparing the VR2 activity of the mixture to a standard. Fusion proteins, such as those made from Fc portion and VR2 polypeptide, as hereinbefore described, can also be used for high-throughput screening assays to identify antagonists for the polypeptide of the present invention (see D. Bennett *et al.*, *J. Mol.*

Recognition, 8:52-58 (1995); and K. Johanson *et al.*, *J. Biol. Chem.*, **270**(16):9459-9471 (1995)).

The polynucleotides, polypeptides and antibodies to the VR2 polypeptides may also be used to configure screening methods for detecting the effect of added compounds on the production of mRNA and polypeptide in cells. For example, an ELISA assay may be constructed for measuring secreted or cell associated levels of polypeptide using monoclonal and polyclonal antibodies by standard methods known in the art. This can be used to discover agents which may inhibit or enhance the production of polypeptide (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues.

Examples of potential polypeptide antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligands, substrates or receptors of the VR2 polypeptide, e g, a fragment of the ligands, substrates or receptors or small molecules which bind to the VR2 polypeptides of the present invention but do not elicit a response, so that the activity of the VR2 polypeptide is prevented.

It will be readily appreciated by the skilled artisan that a VR2 polypeptide may also be used in a method for the structure-based design of a compound that modulates the activity of the VR2 polypeptide by:

(a) determining in the first instance the three-dimensional structure of the VR2 polypeptide;

(b) deducing the three-dimensional structure for the likely reactive or binding site (s) of a modulating compound;

(c) synthesising candidate modulating compounds that are predicted to bind to or react with the deduced binding or reactive site; and

(d) testing whether the candidate compounds are indeed modulators.

It will be further appreciated that this will normally be an iterative process.

Pre-term labour is a major obstetric problem because of the high incidence of neonatal mortality or long-term handicap associated with it.

Approximately 6-10% of all births in the UK are premature in nature and pre-term delivery is responsible for 70-85% of neonatal morbidity and mortality. Currently available drugs are poorly effective and have potentially serious side effects, for the mother and/or foetus. In a further aspect of the present invention, there is therefore provided methods of inducing myometrial relaxation, preventing pre-term labour, stopping labour, for example, in preparation for a Caesarean delivery, and/or treating or preventing dysmenorrhea.

Impotence can be defined literally as a lack of power, in the male, to copulate and may involve an inability to achieve penile erection or ejaculation, or both. More specifically, erectile impotence or dysfunction may be defined as an inability to obtain or sustain an erection adequate for intercourse. Its prevalence is claimed to be between 2 and 7% of the human male population, increasing with age, up to 50 years, and between 18 and 75% between 55 and 80 years of age. In the USA alone, for example, it has been estimated that there are up to 10 million impotent males, with the majority suffering from problems of organic rather than of psychogenic origin. One third of older men receiving medical treatment also has difficulty with erectile function. Risk factors for this disorder include atherosclerosis, hypertension, diabetes mellitus, depression and other psychiatric disorders, hypogonadism, pelvic surgery, kidney failure, multiple sclerosis, stroke, epilepsy, and alcoholism. In a further aspect of the present invention, there is therefore provided methods for the treatment or prevention of erectile dysfunction and/or impotence.

Twenty percent of the adult population suffers from chronic hypertension and 70% of patients with type 2 diabetes are hypertensive. High blood pressure is an important component in the development of atherosclerosis and other illnesses. In a further aspect of the present invention, there is therefore provided methods for the treatment or prevention of hypertension and/or congestive heart failure, inducing diuresis, and/or inhibiting platelet agglutination.

Blood pressure problems (e.g. hypertension and hypertension-induced pre-eclampsia) are one of the most common reasons women are admitted to hospital during pregnancy, particularly during the final trimester. If untreated, pre-eclampsia can lead to eclampsia, which is very dangerous. Pre-eclampsia and eclampsia are the most important causes of death during pregnancy in the UK, USA and Nordic countries. Between 5 and 10% of women in their first pregnancies develop pre-eclampsia. There are also corresponding risks for the foetus. Few, if any, treatments are currently available for this condition and often the pregnant mother is forced to be hospitalised. Early induction of labour is often seen as a necessity in mothers suffering from eclampsia. In a further aspect of the present invention, there is therefore provided methods for the treatment or prevention of pre-eclampsia and/or eclampsia.

Where appropriate, however, it will also be appreciated that a VR2 agonist may be of use in the induction of labour if necessary.

As used herein, the term "treatment" refers both to the treatment and, unless otherwise stated, to prevention or prophylactic therapy to prevent occurrence or recurrence of the aforementioned conditions.

If the activity of the VR2 polypeptide is in excess, several approaches are available. One approach comprises administering to a subject in need thereof an inhibitor compound (antagonist) as hereinabove described, optionally in combination with a pharmaceutically acceptable carrier, in an amount effective to inhibit the function of the VR2 polypeptide, such as, for example, by blocking the binding of ligands, substrates, receptors, enzymes, etc., or by inhibiting a second signal, and thereby alleviating the abnormal condition. In another approach, soluble forms of the VR2 polypeptide still capable of binding the ligand substrate, enzymes, receptors, etc., in competition with endogenous polypeptide may be administered. Typical examples of such competitors include fragments of the VR2 polypeptide.

In still another approach, expression of the gene encoding endogenous VR2 polypeptide can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or externally administered (see, for example, O'Connor, *J. Neurochem.*, (1991) **56**:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton. FL (1988)). Such antisense polynucleotides are designed to comprise the antisense sequence of a polynucleotide encoding a VR2 polypeptide, or a fragment thereof. A VR2 encoding polynucleotide can include a DNA or an RNA, for example a mRNA.

Alternatively, oligonucleotides which form triple helices ("triplexes") with the gene can be supplied (see, for example, Lee *et al.*, *Nucleic Acids Res.*, (1979) **6**:3073; Cooney *et al.*, *Science* (1988) **241**:456; Dervan *et al.*, *Science* (1991) **251**:1360). These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*. Synthetic antisense or triplex oligonucleotides may comprise modified bases or modified backbones. Examples of the latter include methylphosphonate, phosphorothioate or peptide nucleic acid backbones. Such backbones are incorporated in the antisense or triplex oligonucleotide in order to provide protection from degradation by nucleases and are well known in the art. Antisense and triplex molecules synthesised with these or other modified backbones also form part of the present invention.

In addition, expression of the human VR2 polypeptide may be prevented by using ribozymes specific to the human VR2 mRNA sequence. Ribozymes are catalytically active RNAs that can be natural or synthetic (see for example Usman, N, *et al. Curr. Opin. Struct. Biol.*, (1996) **6**(4):527-33). Synthetic ribozymes can be designed to specifically cleave the human VR2 mRNAs at selected positions thereby preventing translation of the human VR2 mRNAs into functional polypeptide. Ribozymes may be synthesised with a natural ribose phosphate backbone and natural bases, as normally found in RNA molecules. Alternatively the ribozymes may be

synthesised with non- natural backbones to provide protection from ribonuclease degradation, for example, 2'-O-methyl RNA, and may contain modified bases.

For treating abnormal conditions related to an under-expression of VR2 and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates a VR2 polypeptide of the present invention, i.e. an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of VR2 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo*. For an overview of gene therapy, see Chapter 20, Gene Therapy and other Molecular Genetic-based Therapeutic Approaches, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996). Another approach is to administer a therapeutic amount of a VR2 polypeptide of the present invention in combination with a suitable pharmaceutical carrier.

In a further aspect, the present invention provides for pharmaceutical compositions comprising a therapeutically effective amount of a VR2 polypeptide, such as the soluble form of a VR2 polypeptide of the present invention, agonist/antagonist peptide, or small molecule compound, in combination with a pharmaceutically acceptable carrier or excipient. Such carriers include, but are not limited to, saline,

buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention. VR2 polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

The composition will be adapted to the route of administration, for instance by a systemic or an oral route. Preferred forms of systemic administration include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if a VR2 polypeptide or other compounds of the present invention can be formulated in an enteric or an encapsulated formulation, oral administration may also be possible. Administration of these compounds may also be topical and/or localised, in the form of salves, pastes, gels, and the like.

It will be appreciated that the amount of a compound of formula (I) required for use in any treatment will vary not only with the particular compounds or composition selected but also with the route of administration, the nature of the condition being treated, and the age and condition of the patient, and will ultimately be at the discretion of the attendant physician. Suitable dosages, however, are in the range of 0.1 to 100 g/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection.

Variations in these dosage levels can be adjusted using standard empirical routines for optimisation, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a VR2 polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

The following definitions are provided to facilitate understanding of certain terms used frequently hereinbefore.

"Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanised antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

"Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated", but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term "polynucleotide" also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons.

"Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications may be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e. peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins.

Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as post-translational processing, or by chemical modification techniques which are well known in the art. Such modifications are well-described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications may occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present to the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from post-translation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, biotinylation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization,

disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination (see, for instance, Proteins-Structure and Molecular Properties, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993; Wolds F., Post-translational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in Post-translational Covalent Modification of Proteins, B. C., Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", *Meth. Enzymol.*, (1990) **182**:626-646 and Rattan *et al.*, "Protein Synthesis; Post-translational Modifications and Aging". *Ann. NY Acad. Sci.*, (1992) **663**:48-62).

"Variant" refers to a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, and deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a

naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity", as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, New York, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM *J. Applied Math.*, 48:1073 (1988). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package (Devereux, J., *et al.*, *Nucleic Acids Res.*, **12**(1):387 (1984)), BLASTP, BLASTN, and FASTA (Atschul, S. F. *et al.*, *J. Molec. Biol.*, **215**:403-410 (1990). The BLAST X program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S., *et al.*, NCBI NLM NIH Bethesda, MD 20894). The well-known Smith Waterman algorithm may also be used to determine identity.

By way of example, a polynucleotide sequence of the present invention may be identical to the reference sequence of SEQ ID NO: 1, that is be 100% identical, or it may include up to a certain integer number of nucleotide alterations as compared to the reference sequence. Such alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. The number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO: 1 by the numerical percent of the respective percent identity (divided by 100) and subtracting that product from said total number of nucleotides in SEQ ID NO: 1, or:

$$n_n \leq x_n - (x_n \cdot y)$$

wherein n_n is the number of nucleotide alterations, x_n is the total number of nucleotides in SEQ ID NO: 1, and y is, for instance, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, etc., and wherein any non-integer product of x_n and y is rounded down to the nearest integer prior to subtracting it from x_n. Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO: 2 may create nonsense. missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

Similarly, a polypeptide sequence of the present invention may be identical to the reference sequence of SEQ ID NO: 2, that is be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the % identity is less than 100%. Such alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said

alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for a given % identity is determined by multiplying the total number of amino acids in SEQ ID NO: 2 by the numerical percent of the respective percent identity (divided by 100) and then subtracting that product from said total number of amino acids in SEQ ID NO: 2, or:

$$n_a \leq x_a - (x_a \cdot y)$$

wherein n_a is the number of amino acid alterations, x_a is the total number of amino acids in SEQ ID NO: 2, and y is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, etc., and wherein any non-integer product of x_a and y is rounded down to the nearest integer prior to subtracting it from x_a.

"Homolog" is a generic term used in the art to indicate a polynucleotide or polypeptide sequence possessing a high degree of sequence relatedness to a subject sequence. Such relatedness may be quantified by determining the degree of identity and/or similarity between the sequences being compared as hereinbefore described. Falling within this generic term are the terms "ortholog", meaning a polynucleotide or polypeptide that is the functional equivalent of a polynucleotide or polypeptide in another species, and "paralog" meaning a functionally similar sequence when considered within the same species.

"Fusion protein" refers to a protein encoded by two, often unrelated, fused genes or fragments thereof. For instance, employing an immunoglobulin Fc region as a part of a fusion protein is advantageous for use in therapy and diagnosis resulting in, for example, improved pharmacokinetic properties. On the other hand, for some uses it would be desirable to be able to delete the Fc part after the fusion protein has been expressed, detected and purified.

The term "effective amount" shall mean that amount of a drug or pharmaceutical agent that will elicit the biological or medical response of a tissue, system, animal or human that is being sought by a researcher or clinician.

Localisation of VR2 (TRPV2) in primate brain was performed as follows:

Materials and Methods

Antibodies

To raise specific polyclonal antisera, rabbits were immunized with a short synthetic peptide specific to the human VR2 C-terminal sequence conjugated to keyhole limpet haemocyanin. Resultant antisera were assessed using enzyme linked immunosorbent assay and dot-blot to evaluate extent and strength of the immune response made to the immunogen. Subsequently, sera were affinity-purified for use in immunohistochemical techniques. For single-, double- and triple-labelling investigations, previously characterized sera specific for vasopressin (VP) and oxytocin (OXY) were obtained from commercial sources.

Immunohistochemistry: Single-labelling using colorimetric and fluorescent detection

10% formal-saline-fixed, paraffin-embedded primate brain sections were dewaxed in xylene, incubated in 0.3% hydrogen peroxide in methanol, rehydrated through an ethanol series and microwaved in citrate buffer, pH 6.0 in order to retrieve antigenicity. Sections were then blocked in 5% normal serum in phosphate buffered saline (PBS)-Triton X-100, prior to overnight incubation at +4°C in affinity-purified antiserum in the presence or absence of 20-fold excess immunizing peptide (for VR2-specific antiserum characterization). Subsequently, sections were washed in PBS and immunoreactivity detected using biotinylated secondary antibodies, followed by Avidin Biotin Complex (ABC) and visualized using

diaminobenzidine/hydrogen peroxide. All single-labelled colorimetric immunohistochemical detection steps were performed on a robotic immunostainer to increase intersection staining consistency.

For fluorescent detection, immunoreactivity was detected using fluorescein isothiocyanate (FITC) conjugated secondary reagents and visualized using confocal microscopy.

Confocal microscopy: double- and triple-labelling

Slides were processed as detailed above before incubation overnight with affinity-purified antisera. Visualization for double- and triple-labelling was carried out as follows: rabbit anti-VR2 primary antibodies were detected using biotinylated goat anti-rabbit secondary antibody, followed by a tertiary layer of Texas Red Streptavidin; guinea pig anti-VP primary antibodies were detected using anti-guinea pig FITC conjugated sera. Double-and triple-labelling studies involving the detection of oxytocin were carried out using both guinea pig- and mouse-anti-oxytocin antibodies. When detecting sera raised in guinea pig (e.g. for double-labeling), a FITC-conjugated anti-guinea pig secondary antibody was used, whilst when using mouse anti-oxytocin antibodies (e.g. for triple-labelling) anti-OXY primary antibodies were detected using a Cy5.5-conjugated anti-mouse secondary antiserum. Sections were mounted in Immu-Mount (Shandon, Pennsylvania, USA) and visualized using a Multi Band Confocal Imaging Spectrophotometer (Leica TCS SP, Wetzlar, Germany).

Results

Single label immunohistochemistry

Using single-labelling immunohistochemistry, VR2-like-immunoreactive material (-ir) was abundantly, yet discretely localized in primate brain. Highly intense VR2-ir was observed in paraventricular nucleus of the hypothalamus (PVN), supraoptic nucleus (SON) and suprachiasmatic nucleus (SCN) (see Figures 2 and 3). These expression

data suggest that VR2 may have neuroendocrine regulatory function(s), as these regions are the neuroanatomical location of oxytocinergic and vasopressinergic neurons, as well as those that express corticotrophin releasing factor (CRF). This was subsequently confirmed using immunohistochemistry using sera specific for oxytocin (OXY) and vasopressin (VP) in sections from the same primates used to investigate VR2 expression.

Double- and triple-labelling confocal microscopy

To gain further understanding of the extent of co-expression of VR2 with OXY and VP, single-, double- and triple-labelling confocal immunohistochemistry was carried out (see Figures 4 and 5). In brief, VR2-ir was shown by double-labelling to be almost entirely restricted to oxytocinergic and vasopressinergic neurons: in both PVN and SON; most, if not all, OXY-positive cells expressed VR2-ir, but not all VR2-ir cells were OXY-positive; likewise, most, if not all, VP-positive cells expressed VR2-ir, but not all VR2-ir cells were VP positive. These data further implicated the involvement of VR2 in hypophyseal function.

To conclusively determine exclusivity of expression of VR2 to OXY- and VP-expressing neurons, triple-labelling confocal immunofluourescence was carried out on PVN, SON, suprachiasmatic nucleus (SCN) and pituitary sections. The resultant data confirmed previous single- and double-labelling investigations. Briefly, cell counts across all 3 regions, PVN, SON and SCN were consistent; approximately 50% of all cells counted were triple labelled for VR2-/VP-/OXY-ir. Except for a very few number of cells, all VR2-ir positive cells were also labelled for either VP-ir and/or OXY-ir, i.e. there were little, if any, singlelabelled VR2-ir cells, no cells labelled for VP-ir alone, and no cells double labelled for VP-ir and OXY-ir alone. These data convincingly indicate involvement of VR2 in hypophyseal functions involving VP and OXY including, anxiety and

depression, diuresis, erectile function, lactation, parturition and sleep, and related behaviours, including schizophrenia.

CLAIMS:

1. The use of a compound selected from:

(a) a VR2 polypeptide;

(b) a compound which modulates the activity of a VR2 polypeptide;

(c) a polynucleotide encoding a VR2 polypeptide; or

(d) an antisense polynucleotide to a polynucleotide encoding a VR2 polypeptide,

for the manufacture of a medicament for treating or preventing pre-term labour, erectile dysfunction, hypertension and/or eclampsia, and associated disorders or schizophrenia.

2. The use according to Claim 1 for treating or preventing pre-term labour, erectile dysfunction, hypertension and/or eclampsia and associated disorders.

3. The use according to Claim 2 for inducing myometrial relaxation, preventing pre-term labour, stopping labour, and/or treating or preventing dysmenorrhea.

4. The use according to Claim 2 for the treatment or prevention of erectile dysfunction and/or impotence.

5. The use according to Claim 2 for the treatment or prevention of hypertension and/or congestive heart failure, inducing diuresis, and/or inhibiting platelet agglutination.

6. The use according to Claim 2 for the treatment or prevention of pre-eclampsia and/or eclampsia.

7. The use according to any one of Claims 1 to 6 wherein the compound which modulates the activity of a VR2 polypeptide is an antagonist.

8. The use according to any one of Claims 1 to 6 wherein the compound is a VR2 polypeptide which comprises a polypeptide having at least 95% identity to the VR2 polypeptide of SEQ ID NO: 2.

9. The use according to Claim 8 wherein the compound is the VR2 polypeptide of SEQ ID NO: 2.

10. The use according to any one of Claims 1 to 6 wherein the compound comprises a polynucleotide encoding a polypeptide having at least 95% identity with the amino acid sequence of SEQ ID NO: 2.

11. The use according to Claim 10 wherein the polynucleotide comprises a polynucleotide having at least 95% identity with the polynucleotide of SEQ ID NO: 1.

12. The use according to Claim 10 or Claim 11 wherein the polynucleotide has the polynucleotide sequence of SEQ ID NO: 1.

13. A method for the treatment of pre-term labour, erectile dysfunction, hypertension and/or eclampsia, and associated disorders or schizophrenia which comprises administration of an effective amount of a compound selected from:

(a) a VR2 polypeptide;

(b) a compound which modulates the activity of a VR2 polypeptide;

(c) a polynucleotide encoding a VR2 polypeptide; or

(d) an antisense polynucleotide to a polynucleotide encoding a VR2 polypeptide,

to a patient in need of such treatment.

14. A method of Claim 13 for the treatment of pre-term labour, erectile dysfunction, hypertension and/or eclampsia, and associated disorders.

15. A method of Claim 14 inducing myometrial relaxation, preventing pre-term labour, stopping labour, and/or treating or preventing dysmenorrhea.

16. A method of Claim 14 for the treatment or prevention of erectile dysfunction and/or impotence.

17. A method of Claim 14 for the treatment or prevention of hypertension and/or congestive heart failure, inducing diuresis, and/or inhibiting platelet agglutination.

18. A method of Claim 14 for the treatment or prevention of pre-eclampsia and/or eclampsia.

19. A method of Claim 14 wherein the compound which modulates the activity of a VR2 polypeptide is an antagonist.

20. A method of Claim 14 wherein the compound is a VR2 polypeptide which comprises a polypeptide having at least 95% identity to the VR2 polypeptide of SEQ ID NO: 2.

21. A method of Claim 14 wherein the compound is the VR2 polypeptide of SEQ ID NO: 2.

22. A method of Claim 14 wherein the compound comprises a polynucleotide encoding a polypeptide having at least 95% identity with the amino acid sequence of SEQ ID NO: 2.

23. A method of Claim 22 wherein the polynucleotide comprises a polynucleotide having at least 95% identity with the polynucleotide of SEQ ID NO: 1.

24. A method of Claim 22 or Claim 23 wherein the polynucleotide has the polynucleotide sequence of SEQ ID NO: 1.

FIGURE 1

Nucleotide and deduced amino acid sequence of human VR2

```
5    CACGAGGCCGACGCGCAGCTGGGAGGAAGACAGGACCCTTGACATCTCCATCTGCACAGA

     GGTCCTGGCTGGACCGAGCAGCCTCCTCCTCCTAGGATGACCTCACCCTCCAGCTCTCCA
                                          M  T  S  P  S  S  S  P

10   GTTTTCAGGTTGGAGACATTAGATGGAGGCCAAGAAGATGGCTCTGAGGCGGACAGAGGA
     V  F  R  L  E  T  L  D  G  G  Q  E  D  G  S  E  A  D  R  G

     AAGCTGGATTTTGGGAGCGGGCTGCCTCCCATGGAGTCACAGTTCCAGGGCGAGGACCGG
     K  L  D  F  G  S  G  L  P  P  M  E  S  Q  F  Q  G  E  D  R
15

     AAATTCGCCCCTCAGATAAGAGTCAACCTCAACTACCGAAAGGGAACAGGTGCCAGTCAG
     K  F  A  P  Q  I  R  V  N  L  N  Y  R  K  G  T  G  A  S  Q

     CCGGATCCAAACCGATTTGACCGAGATCGGCTCTTCAATGCGGTCTCCCGGGGTGTCCCC
20   P  D  P  N  R  F  D  R  D  R  L  F  N  A  V  S  R  G  V  P

     GAGGATCTGGCTGGACTTCCAGAGTACCTGAGCAAGACCAGCAAGTACCTCACCGACTCG
     E  D  L  A  G  L  P  E  Y  L  S  K  T  S  K  Y  L  T  D  S

25   GAATACACAGAGGGCTCCACAGGTAAGACGTGCCTGATGAAGGCTGTGCTGAACCTTAAG
     E  Y  T  E  G  S  T  G  K  T  C  L  M  K  A  V  L  N  L  K

     GACGGAGTCAATGCCTGCATTCTGCCACTGCTGCAGATCGACAGGGACTCTGGCAATCCT
     D  G  V  N  A  C  I  L  P  L  L  Q  I  D  R  D  S  G  N  P
30

     CAGCCCCTGGTAAATGCCCAGTGCACAGATGACTATTACCGAGGCCACAGCGCTCTGCAC
     Q  P  L  V  N  A  Q  C  T  D  D  Y  Y  R  G  H  S  A  L  H

     ATCGCCATTGAGAAGAGGAGTCTGCAGTGTGTGAAGCTCCTGGTGGAGAATGGGGCCAAT
35   I  A  I  E  K  R  S  L  Q  C  V  K  L  L  V  E  N  G  A  N

     GTGCATGCCCGGGCCTGCGGCCGCTTCTTCCAGAAGGGCCAAGGGACTTGCTTTTATTTC
     V  H  A  R  A  C  G  R  F  F  Q  K  G  Q  G  T  C  F  Y  F

40   GGTGAGCTACCCCTCTCTTTGGCCGCTTGCACCAAGCAGTGGGATGTGGTAAGCTACCTC
     G  E  L  P  L  S  L  A  A  C  T  K  Q  W  D  V  V  S  Y  L

     CTGGAGAACCCACACCAGCCCGCCAGCCTGCAGGCCACTGACTCCCAGGGCAACACAGTC
     L  E  N  P  H  Q  P  A  S  L  Q  A  T  D  S  Q  G  N  T  V
45

     CTGCATGCCCTAGTGATGATCTCGGACAACTCAGCTGAGAACATTGCACTGGTGACCAGC
     L  H  A  L  V  M  I  S  D  N  S  A  E  N  I  A  L  V  T  S

     ATGTATGATGGGCTCCTCCAAGCTGGGGCCCGCCTCTGCCCTACCGTGCAGCTTGAGGAC
50   M  Y  D  G  L  L  Q  A  G  A  R  L  C  P  T  V  Q  L  E  D

     ATCCGCAACCTGCAGGATCTCACGCCTCTGAAGCTGGCCGCCAAGGAGGGCAAGATCGAG
     I  R  N  L  Q  D  L  T  P  L  K  L  A  A  K  E  G  K  I  E

55   ATTTTCAGGCACATCCTGCAGCGGGAGTTTTCAGGACTGAGCCACCTTTCCCGAAAGTTC
```

```
I  F  R  H  I  L  Q  R  E  F  S  G  L  S  H  L  S  R  K  F

ACCGAGTGGTGCTATGGGCCTGTCCGGGTGTCGCTGTATGACCTGGCTTCTGTGGACAGC
T  E  W  C  Y  G  P  V  R  V  S  L  Y  D  L  A  S  V  D  S

TGTGAGGAGAACTCAGTGCTGGAGATCATTGCCTTTCATTGCAAGAGCCCGCACCGACAC
C  E  E  N  S  V  L  E  I  I  A  F  H  C  K  S  P  H  R  H

CGAATGGTCGTTTTGGAGCCCCTGAACAAACTGCTGCAGGCGAAATGGGATCTGCTCATC
R  M  V  V  L  E  P  L  N  K  L  L  Q  A  K  W  D  L  L  I

CCCAAGTTCTTCTTAAACTTCCTGTGTAATCTGATCTACATGTTCATCTTCACCGCTGTT
P  K  F  F  L  N  F  L  C  N  L  I  Y  M  F  I  F  T  A  V

GCCTACCATCAGCCTACCCTGAAGAAGCAGGCCGCCCCTCACCTGAAAGCGGAGGTTGGA
A  Y  H  Q  P  T  L  K  K  Q  A  A  P  H  L  K  A  E  V  G

AACTCCATGCTGCTGACGGGCCACATCCTTATCCTGCTAGGGGGGATCTACCTCCTCGTG
N  S  M  L  L  T  G  H  I  L  I  L  L  G  G  I  Y  L  L  V

GGCCAGCTGTGGTACTTCTGGCGGCGCCACGTGTTCATCTGGATCTCGTTCATAGACAGC
G  Q  L  W  Y  F  W  R  R  H  V  F  I  W  I  S  F  I  D  S

TACTTTGAAATCCTCTTCCTGTTCCAGGCCCTGCTCACAGTGGTGTCCCAGGTGCTGTGT
Y  F  E  I  L  F  L  F  Q  A  L  L  T  V  V  S  Q  V  L  C

TTCCTGGCCATCGAGTGGTACCTGCCCCTGCTTGTGTCTGCGCTGGTGCTGGGCTGGCTG
F  L  A  I  E  W  Y  L  P  L  L  V  S  A  L  V  L  G  W  L

AACCTGCTTTACTATACACGTGGCTTCCAGCACACAGGCATCTACAGTGTCATGATCCAG
N  L  L  Y  Y  T  R  G  F  Q  H  T  G  I  Y  S  V  M  I  Q

AAGGTCATCCTGCGGGACCTGCTGCGCTTCCTTCTGATCTACTTAGTCTTCCTTTTCGGC
K  V  I  L  R  D  L  L  R  F  L  L  I  Y  L  V  F  L  F  G

TTCGCTGTAGCCCTGGTGAGCCTGAGCCAGGAGGCTTGGCGCCCCGAAGCTCCTACAGGC
F  A  V  A  L  V  S  L  S  Q  E  A  W  R  P  E  A  P  T  G

CCCAATGCCACAGAGTCAGTGCAGCCCATGGAGGGACAGGAGGACGAGGGCAACGGGGCC
P  N  A  T  E  S  V  Q  P  M  E  G  Q  E  D  E  G  N  G  A

CAGTACAGGGGTATCCTGGAAGCCTCCTTGGAGCTCTTCAAATTCACCATCGGCATGGGC
Q  Y  R  G  I  L  E  A  S  L  E  L  F  K  F  T  I  G  M  G

GAGCTGGCCTTCCAGGAGCAGCTGCACTTCCGCGGCATGGTGCTGCTGCTGCTGCTGGCC
E  L  A  F  Q  E  Q  L  H  F  R  G  M  V  L  L  L  L  L  A

TACGTGCTGCTCACCTACATCCTGCTGCTCAACATGCTCATCGCCCTCATGAGCGAGACC
Y  V  L  L  T  Y  I  L  L  L  N  M  L  I  A  L  M  S  E  T

GTCAACAGTGTCGCCACTGACAGCTGGAGCATCTGGAAGCTGCAGAAAGCCATCTCTGTC
V  N  S  V  A  T  D  S  W  S  I  W  K  L  Q  K  A  I  S  V

CTGGAGATGGAGAATGGCTATTGGTGGTGCAGGAAGAAGCAGCGGGCAGGTGTGATGCTG
L  E  M  E  N  G  Y  W  W  C  R  K  K  Q  R  A  G  V  M  L

ACCGTTGGCACTAAGCCAGATGGCAGCCCGGATGAGCGCTGGTGCTTCAGGGTGGAGGAG
T  V  G  T  K  P  D  G  S  P  D  E  R  W  C  F  R  V  E  E
```

GTGAACTGGGCTTCATGGGAGCAGACGCTGCCTACGCTGTGTGAGGACCCGTCAGGGGCA
V N W A S W E Q T L P T L C E D P S G A

GGTGTCCCTCGAACTCTCGAGAACCCTGTCCTGGCTTCCCCTCCCAAGGAGGATGAGGAT
G V P R T L E N P V L A S P P K E D E D

GGTGCCTCTGAGGAAAACTATGTGCCCGTCCAGCTCCTCCAGTCCAACTGATGGCCCAGA
G A S E E N Y V P V Q L L Q S N *

TGCAGCAGGAGGCCAGAGGACAGAGCAGAGGATCTTTCCAACCACATCTGCTGGCTCTGG

GGTCCCAGT

FIGURE 2

Single-label colorimetric immunohistochemistry showing highly abundant expression of VR2-ir in primate supraoptic nucleus (SO) and paraventricular nucleus of the hypothalamus (PVN)

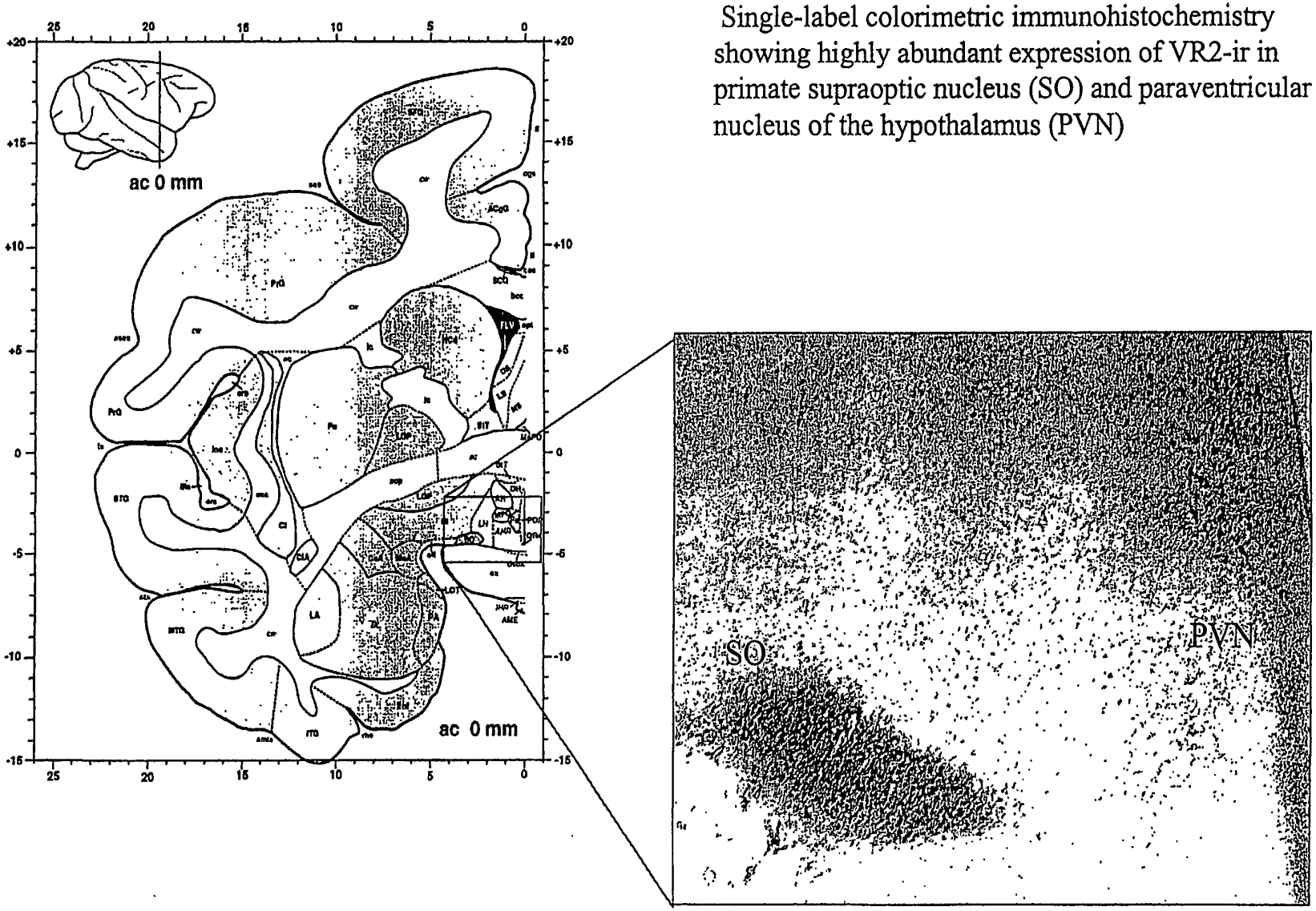
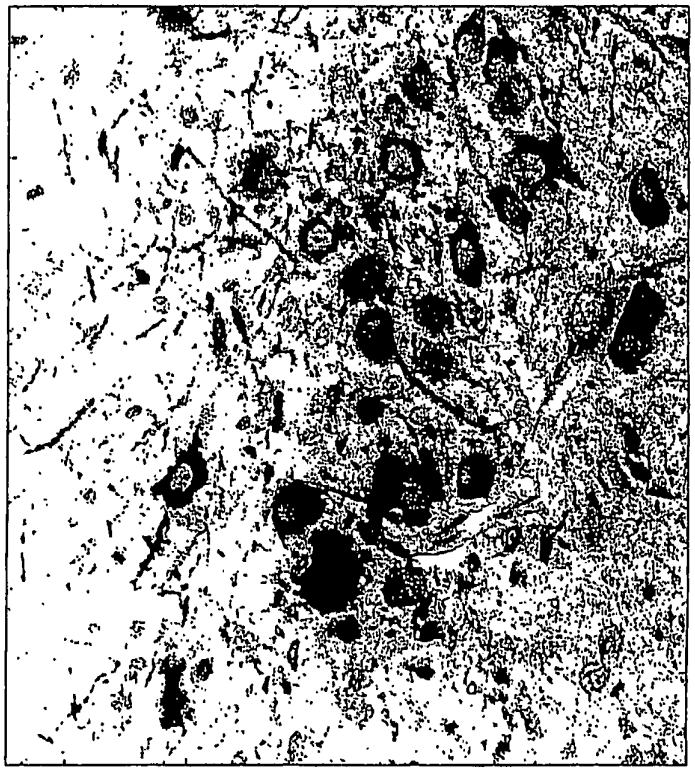


FIGURE 3

Localization of VR2-ir in primate pituitary and suprachiasmatic nucleus

Pituitary



Suprachiasmatic nucleus

FIGURE 4

Regional co-expression of VR2-ir, oxytocin-ir and vasopressin-ir distribution in primate hypothalamic paraventricular nucleus

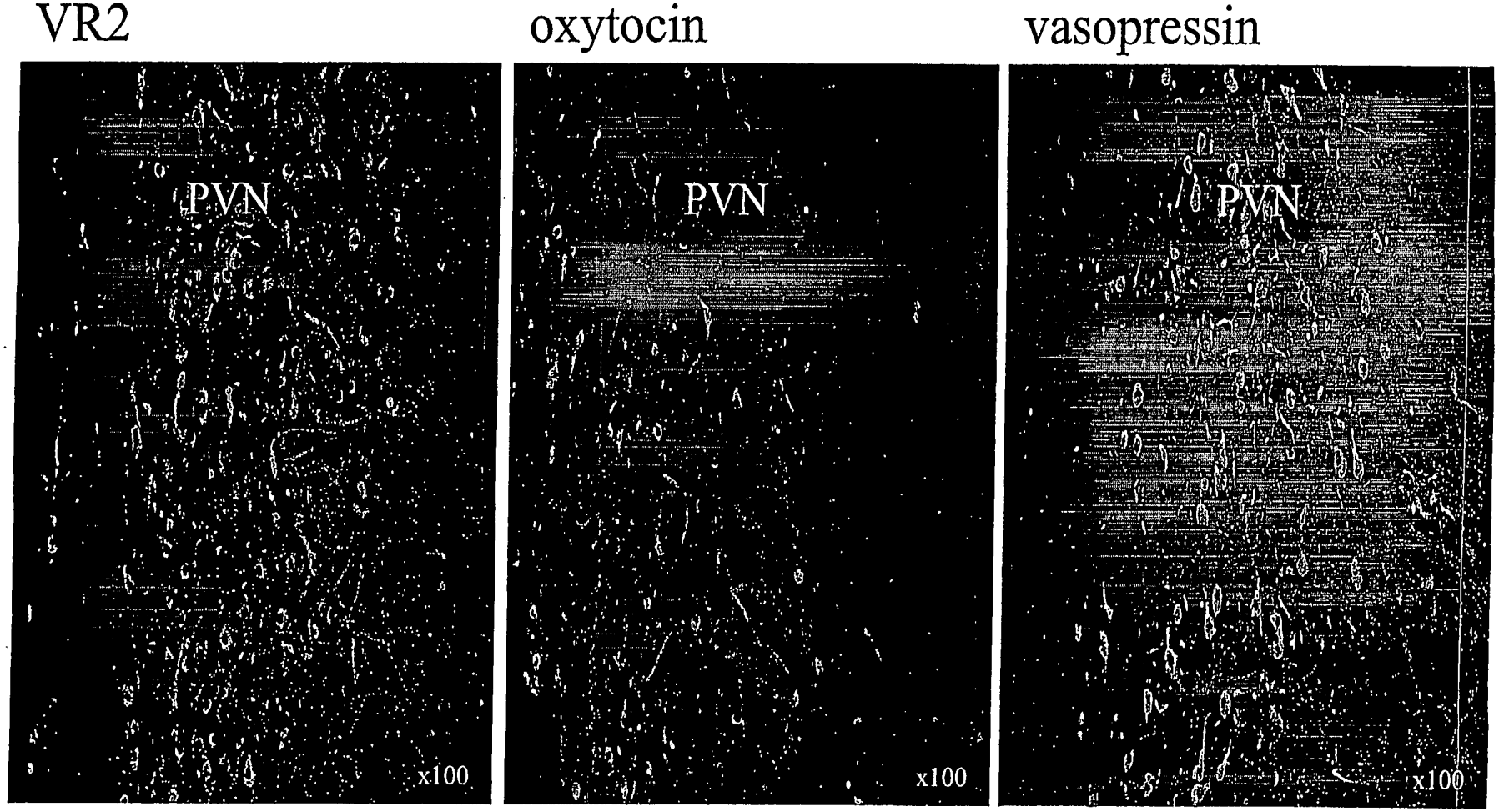


FIGURE 5

Regional co-expression of VR2-ir, oxytocin-ir and vasopressin-ir distribution in primate supraoptic nucleus (SON)